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PATENT
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AMENDMENTS TO THE CLAIMS

Examiner 1657

Please enter the following amendments to the claims:

1-152. **(Cancelled)**

153. **(New)** A method for producing a substantially pure, recombinant, glycosylated erythropoietin (EPO) that has *in vivo* activity, including erythropoiesis, comprising:

a) infecting insect cells that grow in serum-free media with a baculovirus expression system comprising a recombinant baculovirus that comprises DNA coding for EPO such that the recombinant EPO is expressed;

b) culturing the infected insect cells in serum-free media; and

c) purifying the recombinant EPO to 95% or greater purity,

whereby the substantially pure, recombinant, glycosylated EPO that has *in vivo* activity, including erythropoiesis, is produced.

154. **(New)** The method of claim 153, wherein the insect cells are *Spodoptera frugiperda* cells.

155. **(New)** The method of claim 153, wherein the insect cells are *Spodoptera frugiperda* SF900+ cells.

156. **(New)** The method of claim 153, wherein the substantially pure, recombinant, glycosylated EPO has activity of 200,000 U/mg protein.

157. **(New)** The method of claim 153, wherein the substantially pure, recombinant, glycosylated EPO has activity of between 200,000 U/mg protein and 500,000 U/mg protein.

158. **(New)** The method of claim 153, wherein the substantially pure, recombinant, glycosylated EPO has activity of 500,000 U/mg protein.

159. **(New)** The method of claim 153, wherein the infecting of the insect cells with the recombinant baculovirus, the culturing of the insect cells, or both is in an apparatus for growing cells, wherein the apparatus comprises:

(a) at least one bioreactor for cell culture;

(b) at least one vessel for culture media;

whereby the bioreactor and vessel are in fluid communication, and

wherein the bioreactor, vessel, or both are optionally stirred;

(c) a dialysis means for circulating culture media, cell culture, or both,

whereby there is a first cell culture loop between the bioreactor and the dialysis means and a second media replenishment loop between the vessel and the dialysis means;

(d) in-operation dialysis between the culture media and the cell culture;

(e) at least one means for delivery of oxygen comprising a hollow fiber filter oxygenator, whereby the oxygen is delivered directly to cells in a circulating loop of cells before cell entry into the hollow fiber filter.

160. **(New)** The method of claim 159, wherein in the apparatus the means for delivery of oxygen comprises at least one or more of the following:

means for in-line sparging;

means for delivery of at least one oxygen-containing compound that releases dissolved oxygen into cell culture;

means for delivery of oxygen positioned upstream of input of circulating cell culture returning to the bioreactor;

means for delivery of oxygen providing an average dissolved oxygen concentration of about 60%;

means for delivery of oxygen providing an average dissolved oxygen concentration of greater than about 40%; and,

means for delivery of oxygen providing an average dissolved oxygen concentration between about 30% and about 90%, between about 40% and about 80%, or between about 50% and about 70%.

161. **(New)** The method of claim 160, wherein in the apparatus the means for delivery of oxygen comprises at least one or more of the following:

means for in-line sparging;

means for delivery of at least one oxygen-containing compound that releases dissolved oxygen into cell culture; and,

means for delivery of oxygen positioned upstream of input of circulating cell culture returning to the bioreactor.

162. **(New)** The method of claim 160, wherein in the apparatus the dialysis means comprises at least one semi-permeable membrane, at least one means for delivery of oxygen into the cell culture loop, or both.

163. **(New)** The method of claim 160, wherein the apparatus further comprises one or more of the following:

- means for measuring physical parameters of the cell culture or the cell culture media;
- means for measuring chemical parameters of the cell culture or the culture media;
- means for measuring dissolved oxygen concentration;
- means for measuring pH;
- means for measuring pH and dissolved oxygen concentration;
- means for measuring temperature;
- means for measuring cell density or amount of cells;
- means for adjusting physical parameters of the cell culture or the cell culture media in response to data from the measuring means;
- means for adjusting chemical parameters of the cell culture or the culture media in response to data from the measuring means;
- means for adjusting dissolved oxygen concentration;
- means for adjusting pH;
- means for adjusting temperature;
- means for adjusting dissolved carbon dioxide concentration; and
- means for adding a vector in response to a cell density or cell amount measurement.

164. **(New)** The method of claim 160, wherein in the apparatus pH is measured, and in response to the pH measurement, dissolved carbon dioxide concentration is adjusted.

165. **(New)** The method of claim 160, wherein in the apparatus dissolved oxygen concentration is measured, and in response to the dissolved oxygen measurement, the dissolved oxygen concentration is adjusted.

166. **(New)** The method of claim 160, wherein in the apparatus dissolved oxygen concentration is measured, pH is set to a desired level, and carbon dioxide is adjusted when pH varies from the desired level, whereby the dissolved oxygen concentration varies periodically as a function of time.

167. **(New)** The method of claim 160, wherein in the apparatus the dissolved oxygen concentration is measured, and the measurement varies from 30% to 90%, from 40% to 80%, from 50% to 70%, or averages about 60%.

168. **(New)** The method of claim 160, wherein in the apparatus the dissolved oxygen concentration is measured, and the measurement varies from high value to low value over about 10 to about 30 minutes or over about 20 minutes.

169. **(New)** The method of claim 160, wherein in the apparatus the dissolved oxygen concentration is measured, and a plot of the dissolved oxygen concentration measurement as a function of time comprises a sin wave.